

# Methane and carbon dioxide fluxes in wetland mesocosms: Relationships to hydrology and soils

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## Introduction

Wetlands perform complex and important biogeochemical functions in landscapes, including the production, sequestration and release of carbon compounds or gases. As a general rule, wetlands act as both sinks for atmospheric carbon dioxide and sources of the greenhouse gas methane (Bouchard and Cochran, 2002; Whalen, 2005). In addition to climate, hydrology and soils play major roles in determining the nature of wetland carbon dynamics, in both natural and created or restored ecosystems. Riparian wetlands, located within the original flood zone of rivers and streams, receive water in pulses during floods. Between floods, these wetlands may dry out or become partially unsaturated. Numerous non-riverine wetland ecosystems, including prairie potholes (van der Valk and Pederson, 2003), impounded marshes (Johnson Randall and Foote, 2005), vernal pools (Brooks and Hayashi, 2002), and swamps (Carter et al., 1994) experience a dynamic hydroperiod defined by seasonal or annual patterns of inundation and drying. These water level changes are driven by precipitation, runoff and evapotranspiration, in combination with the water table location, the position of the wetland in the landscape, and the nature of the substrate underlying the ecosystem (Carter, 1996). Periodic drawdowns of the water table during the growing season, such as often occurs in natural or restored riparian wetlands, have been demonstrated to reduce methane flux significantly on the ecosystem scale (Altor and Mitsch, in press). Flood pulses have been both positively (Sommer et al., 2001, Ahearn et al., 2006) negatively (Burke et al., 1999) and neutrally (Megonigal et al., 1997) correlated with primary productivity, and it is recognized that gradients of subsidies (e.g. nutrients, sediments) and stresses (e.g. anoxia, dessication, turbulence) interact with biological processes in ecosystems that receive flood pulses (Odum et al., 1979; Burke et al., 1999). The successional stage of riparian ecosystems in part determines whether flood pulses act as a subsidy or stress. Productivity in early successional systems dominated by phytoplankton and emergent macrophytes may be enhanced by flood pulses (Ahearn et al., 2006), while later successional stages dominated by trees may be stressed (Burke et al., 1999) or neutrally affected by these pulses. In all cases, spatial and biogeochemical heterogeneity will generally lead to a gradient of responses to flooding and dry down within wetland ecosystems (Mitsch, 1988; Megonigal et al., 1997).

Major biogeochemical functions in wetlands take place in

the context of the soil or substrate that forms the foundation of the system. When wetlands are constructed in areas lacking hydric soils, soils that have never been flooded, or at least not recently so, are subjected to prolonged saturated conditions. Among the many changes that can occur in soils upon flooding are reduction and mobilization of metal cations (particularly  $\text{Fe}^{3+}$  and  $\text{Mn}^{4+}$ ) and nutrient anions (eg  $\text{NO}_3^-$ ), anaerobic production and release of methane ( $\text{CH}_4$ ) and other reduced gases (e.g.  $\text{H}_2\text{S}$ ), gradual accumulation of refractory organic matter, and changes in microbial community composition (Vepraskas and Faulkner, 2001; Craft, 2001). These changes occur at varying time scales, depending on water chemistry and the structure and chemical composition of the soil prior to flooding. The development of hydric soil characteristics including mottles, oxidized pore linings and redox depletions is an important indication that wetland hydrology is present (NAS, 1995).

Vegetation plays a variety of roles in wetland carbon cycling. Besides photosynthetic uptake of atmospheric  $\text{CO}_2$ , some wetland plant species transport  $\text{O}_2$  from the atmosphere to the rhizosphere (root zone), and deliver gases from the soil to the atmosphere. Pressurized ventilation of methane and other gases, in which pressure and temperature differentials drive convective flows between the atmosphere and soil through vascular plant tissue, have been demonstrated for a variety of emergent and submerged plant species (Schütz et al., 1991; Brix et al., 1996; Große, 1996). When methane is being released to the atmosphere by pressure-driven convection, highest rates of gas flux generally occur during midday when light intensity and temperature are at a maximum (Whiting and Chanton, 1996). Some researchers have postulated that a consistent proportion of  $\text{CO}_2$  taken up by wetland plants may be returned to the atmosphere as  $\text{CH}_4$  (Whiting and Chanton, 1993). Vegetation provides the foundation for autochthonous carbon inputs into wetlands, carbon that can contribute to both the stable, sequestered pool and the labile, microbially-active pool. The objectives of this study were to examine relationships between pulsed vs. continuously inundated hydrology, hydric vs. non-hydric soils, vegetation, methane and carbon dioxide fluxes in replicated wetland mesocosms. The utility of mesocosm and microcosm experiments to test parameters of restoration design has been recognized by other researchers (Calloway et al., 1997; Catallo and Junk, 2003).

In this research we examined patterns of methane flux from hydric and non-hydric soils under continuously

inundated (steady-flow) treatments and periodically flooded and dried (pulsed) conditions. Soil parameters considered to be relevant to methane flux were examined, including total carbon, nitrogen and organic matter contents, Munsell hue, value and chroma, and labile carbon as determined by cold and hot water extractions. Temporal patterns in methane and CO<sub>2</sub> flux were monitored to evaluate primary productivity and the influence of vegetation on methane flux.

## Methods

### Site description

The study was conducted at the Schiermeier Olentangy River Wetland Research Park, on the campus of the Ohio State University, Columbus, Ohio USA. The site houses an outdoor mesocosm compound that consists of four sets of twenty 540 L high-density black plastic tubs, buried in the ground. Other research conducted using the mesocosms on this site includes investigations into the effects of hydric vs. non-hydric soils on growth of wetland vegetation (Nairn, 1996), growth of *Typha latifolia* and *Schoenoplectus tabernaemontani* under conditions of nutrient enrichment (Svengsok and Mitsch, 2001), scaling issues and use of flue gas desulfurization material as a wetland liner (Ahn and Mitsch, 2002a,b), and response of vegetation to pulsing vs. steady-flow hydrology (Anderson and Mitsch, 2005). The mesocosm tubs are designed to be flow-through systems, with a drainage outlet drilled into the end of each tub. Drainage also occurs through French drains that discharge water after it has seeped through the soil profile. Water levels are controlled using standpipes of various heights placed into the French drains (Figure 1).

The mesocosm compound was designed to have access to water from the adjacent Olentangy River via a pump that fed from the main pumping line that waters two 1-ha experimental wetlands on the site. However, this pump was disabled during the initiation of this study, and groundwater was used as a result. Five sets of paired groundwater and river water samples were taken to verify that the groundwater did not contain toxic concentrations of metals.

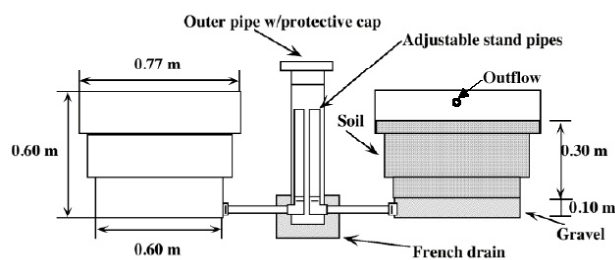


Figure 1. Schematic diagram of mesocosm tub design, illustrating tub dimensions, French drains, stand pipes for adjusting water level, and outflow opening.

### Study design

Twenty mesocosm tubs, containing rinsed pea gravel to a depth of 10 cm, were filled with approximately 35 cm of soil in autumn, 2003. Half of the tubs received non-hydric soil generated from excavation of a building site on the OSU campus, and half of the tubs received hydric soil removed with a backhoe from the upper 10 cm of an onsite wetland that was created in 1997 (the Billabong). The hydric soils were taken from an area of the Billabong that was sparsely vegetated, and were examined for Munsell hue, value and chroma prior to excavation to verify that they met hydric criteria. The non-hydric soils were classified as Ross silt loam - mixed, mesic Cumulic Hapludolls. The Ross series are well-drained, moderately-permeable soils originating from alluvium (SCS, 1980). The type of soil each tub would receive (hydric vs. non-hydric) was chosen randomly, the only criteria being that ten tubs would receive each soil type.

Soils were allowed to settle until spring 2004 when each tub was planted with three rhizomes each of *Sparganium eurycarpum* Engelm and *Schoenoplectus tabernaemontani* K.C. Gmel, as well as seeds of *Leersia oryzoides* L. Sw. Each of these plant species had been dominant in the onsite experimental wetlands in 2003.

After planting the mesocosms, all tubs were maintained under saturated conditions throughout the 2004 growing season in order to allow vegetation to establish. No weeding was conducted; non-planted species were considered part of the potential plant community for each soil type. In March 2004, the first set of soil cores was taken, and the second was taken in March 2005. Hydrologic treatments were assigned randomly to the mesocosms, within soil types. Five tubs with each soil type were assigned pulsed hydrology, and five were assigned steady-flow hydrology, resulting in four treatments of five tubs each. Gas sampling was initiated in April 2005, and continued through September 2005.

### Soil sampling

In March 2004, three soil cores were taken from each mesocosm using a stainless steel soil corer, 2 cm i.d. pushed in as deep as possible. The length of each core retrieved was recorded, and Munsell color, hue and chroma were determined in the field for the matrix and for redoximorphic features. Cores were taken near the center of the tubs, at each end and middle. The stainless steel corer was rinsed and dried between samples. Because the soil was not naturally stratified, having been shoveled into the tubs, cores were not analyzed by depth interval. Instead, the lengths of the three cores taken from each tub were measured, combined into one plastic bag and stored in a cooler (4°C) until further analysis. These soils were dried at 150°C in the laboratory until constant weight to determine bulk density from the combined volume of the three cores per tub. The soil samples were then ground in a mortar and pestle and sieved to <2 mm diameter. Replicate subsamples of approximately 5 g were measured into 10-ml porcelain crucibles. In order to

determine whether carbonates were present, one of each subsample was treated with 10N hydrochloric acid (fizzing indicates that soil contains carbonates), and samples were dried at 105°C until constant weight (.001 g) as measured on a Mettler balance. The samples were then combusted in a Fisher Scientific Isotemp forced-draft furnace for three hours, in one hour intervals, at 550°C. After each hour, combusted soils were cooled to 105°C, placed in a dessicator for approximately 30 minutes, and reweighed on the Mettler balance to determine the percent soil organic matter content (SOM) by loss on ignition. Soils were combusted in one hour intervals in order to determine how much change in weight, if any, took place over a three-hour combustion period. A subsample of soil from each mesocosm was analyzed for total carbon and nitrogen content by combustion in an Elementar America's VarioMAX analyzer at Star Lab, Wooster, OH.

A second set of soil cores was taken in March 2005 using the same soil corer, after the mesocosms were saturated continuously for one year. Three cores were taken from each tub, in the center and near each end. One 2 cm section was cut from each core and placed in a plastic bag for laboratory analysis. If there was a visible difference in texture or color along the length of the core, a 2 cm section was taken from each portion. The soil samples from each mesocosm were not combined as they had been in 2004. Soil samples were kept in the shade and brought into the laboratory within one hour of collection. Each of the three or more samples from each tub was analyzed separately using the methods described above for bulk density, and averaged together to obtain one bulk density value for each mesocosm.

Water extractable organic matter (WEOM) < 0.45 µm diameter was determined by sequential cold and hot water extractions on each of the 2005 soil samples, after Nguyen (2000). WEOM in this size class (dissolved) is that fraction considered to be potentially available to microbes (Zsolnay, 2003), because microbial metabolism and physical processes are dependent upon an aquatic environment (Marschner and Kalbitz, 2003). WEOM is generally considered to be labile (Sparling et al., 1998; Chantigny, 2003). Two grams of dry soil were combined with 30 ml deionized water (20°C) in 50 ml centrifuge tubes, placed horizontally on the shaker tray of a controlled temperature Precision Reciprocal Shaking Bath Model 25 and shaken at 120 rpm for 18 hours. Samples were then centrifuged at 2600 rpm for 15 minutes, and the supernatant was filtered through sterile 0.45 µm Whatman polyethersulfone membrane syringe filters. Filtrate was analyzed for total carbon and total inorganic carbon on a Shimadzu TOC5050A with ASI5000A autosampler. 30 ml of deionized water was then added to each tube, and the slurries were shaken at 120 rpm for 18 hours at 80°C. Each sample was centrifuged, filtered and analyzed as above, for hot-water extractable carbon. 20% of the soil samples were tested in duplicate for both cold and hot-water extractable organic carbon. Blank samples of deionized water were analyzed for background concentrations of TC and TIC as well. WESOM values were obtained by subtracting TIC

from TC, and multiplying by the van Bemmelen organic matter factor 1.72, and dividing by 2 because 2 grams of soil were used for the extraction. Values thus obtained were mg WESOM kg<sup>-1</sup> soil.

## Hydrology

The experimental hydrology was established in the mesocosms beginning on April 1, 2005. A gravity-feed system for delivering water from elevated 450 gal (1700 L) tanks through PVC piping was used initially to establish flow to the tubs. Flow valves at the inlet to each mesocosm were adjusted until the desired flow rate was achieved in each tub, as measured with a graduated cylinder and stopwatch. This approach was used for the first two weeks, during which time it became apparent that the accuracy of the flows was not reliable because of changing head in the elevated tanks and variable pressures within the PVC tubes. Beginning April 18, a hose attached directly to a groundwater pump was used to deliver water to each tub. The flow rate from the hose was measured and duration of flow to each tub was timed in order to calculate flow volume, and ensure that approximately the same cumulative volume was delivered to all tubs over the course of each month. Steady flow tubs received water daily, except when precipitation was adequate to maintain inundated conditions, and pulsed tubs received the same amount of water as steady flow tubs, but at higher rates ("flood pulses") and for fewer days. The objectives for each hydrologic treatment were to 1) maintain saturated or inundated conditions at all times for steady-flow tubs, and 2) create a minimum of one cycle of alternating inundated/dry conditions each month in pulsed tubs. Daily temperature and precipitation dynamics determined how many inundated/dry cycles were possible in pulsed mesocosms in a given month.

Porewater was sampled from each mesocosm standpipe on two occasions (June 1 and August 4, 2005), to examine nutrient, organic and inorganic carbon content. Water that flowed out of the mesocosms through the French drain standpipes first seeped through the mesocosm's soil profile. The volume of each standpipe was calculated, and that amount of water was removed and discarded back into the mesocosm before the sample was collected, to ensure that the water collected for porewater samples had not been stagnant in the pipes, but rather was emerging freshly from infiltration through the soil. Porewater samples were collected using a rubber suction bulb attached to Tygon tubing, into 0.5 L acid-washed Nalgene bottles, and stored at 4°C until analysis. Each 500 ml sample was split into 100 ml subsamples for analysis of total Kjeldahl nitrogen (TKN), nitrate-nitrite, total phosphorus (TP), and total inorganic and organic carbon. Nutrient analyses were performed on a Lachat QuikChem IV; total and inorganic carbon were analyzed on the Shimadzu TOC 5050A.

## Gas sampling

Gas sampling was conducted using non-steady-state chambers designed after Klingner et al. (1994) and Altor



and Mitsch (in press). Using a jigsaw, the bottoms were removed from opaque, 20 x 14 inch rectangular plastic tubs (51 by 36 cm). It was important that the tubs have flat sides, because ridges would make it difficult to make clean cuts with the jigsaw. Transparent, 4-mil polyethylene bags (approximately 1.5 m tall) were attached to the plastic bases using weatherproof transparent tape applied inside and outside of the bag. The top of each bag was cut open, and the bags were kept rolled down around the chamber bases between sampling events. One plastic base, with bag attached, was inserted 3-5 cm into the soil in the center of each mesocosm tub, in April of 2005. A knife was used to cut into the soil around the border of the tub base, which facilitated pushing the base into the soil. Once in place, chamber bases were not moved from the tubs for the remainder of the study. Frames made from 1.5 inch dia PVC were installed inside the base of each mesocosm tub, with one PVC leg next to the center of each of the walls of the base. Each leg was inserted approximately 20 cm into the soil, leaving 130 cm height above ground. A rectangular PVC support, with a wire for attaching a thermometer, was placed onto the tops of the legs to complete the frame. These frames served as supports for the bags, and were left in place throughout the study.

During the gas sampling process, the bags were rolled up around the chamber frames, and sealed at the top with 0.5 cm diameter rubber bands. The top of each bag was affixed with a grey butyl rubber sampling port and 2-m Tygon tubing for equilibrating the chamber with atmospheric pressure. Sampling was conducted one day before flood pulses were delivered to pulsed tubs, and one day after, as well as numerous occasions in between flood pulses. Five gas samples were collected from the headspace of each chamber over 20-30 minutes, into pre-evacuated 10 ml autosampler vials. Sampling times consisted of morning (between 7:30-10:30), afternoon (between 12:30-4:30) and after dark. With two field workers, each sampling period took approximately 1.5 hours to complete. The mesocosms at which sampling was started were chosen randomly each sampling day.

Environmental parameters measured in each mesocosm during gas sampling included soil temperature at 5 and 10 cm depths, temperature within the chamber when each gas sample was withdrawn, and water level. Visual estimates of percent cover of each plant species were recorded approximately two times per month, on or close to sampling days. Gas samples were analyzed on a Shimadzu GC 14A equipped with an HTA Autosampler, with a thermal conductivity detector (TCD) and flame ionization detector (FID) in series. A 1.8 m Porapak-Q column was used for sample separation, with helium (approximately 25 ml min<sup>-1</sup>) as the carrier gas. The GC oven and injection temperatures were maintained at 40°C; detector temperatures were 200°C (TCD) and 150°C (FID). Four-point calibration curves were prepared for each GC run using Matheson gas standards. The calibration curves consisted of 5, 10, 15 and 20 ppm CH<sub>4</sub>, with ultrapure N<sub>2</sub> as the balance, and 250, 500, 750

and 1000 ppm CO<sub>2</sub> balanced with helium. Check standards were injected during each run to verify consistency of the analysis. Gas samples were stored at 4°C until analysis, and were analyzed within one week of collection.

### Data analysis

Soil carbon and nitrogen contents of upland and hydric soils (determined before establishing hydrology in the mesocosms) were compared with paired t-tests. Bulk densities, soil organic matter and dissolved carbon in porewater for each soil type, were analyzed for normality and compared with t-tests for unrelated samples, assuming equal variance. Time of combustion for SOM (1 hr v. 2 hr v. 3 hr) was also compared, separately for hydric and non-hydric soils, using t-tests for equal variance. Percent C and N for hydric and non-hydric soils were analyzed with ANOVA or Mann-Whitney tests. Cold and hot water extractable organic matter content for each treatment was analyzed for normality with the Kolmogorov-Smirnov test, and for homogeneity of variance with the Levene Statistic. Mean values of CWEOM AND HWEOM between treatments were compared with one-way ANOVA. T-tests assuming unequal variance were used to compare the total amount of water delivered to each steady flow and pulsed mesocosm each month. All statistical analyses assumed a confidence interval of 95% ( $\alpha = 0.05$ ).

Concentration by volume of methane and carbon dioxide in each sample, determined by gas chromatography, were converted to flux rates (mg CH<sub>4</sub>-C and mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>), corrected for chamber volume and temperature (Healy et al., 1996). Regressions were performed on each flux rate in Microsoft Excel™ to determine linearity of flux. CH<sub>4</sub>-C flux rates with correlations and R<sup>2</sup> < 0.88 were considered to be zero when individual measurements varied less than 1 ppm. If R<sup>2</sup> < 0.88 and CH<sub>4</sub> concentrations varied by more than 1 ppm over the sampling period, the flux rate was discarded. CO<sub>2</sub>-C flux rates with R<sup>2</sup> < 0.88 were discarded. Therefore, only linear positive, negative or zero flux rates were used in the analyses; over 99% of linear flux rates had R<sup>2</sup> > 0.90. Where removing a sample corrected a poor correlation to > 0.90, the sample was eliminated from the calculation (Holland et al., 1999).

Methane fluxes were analyzed according to the four treatments: non-hydric soil with pulsed hydrology, non-hydric soil with steady-flow hydrology, hydric soil with pulsed hydrology, and hydric soil with steady-flow hydrology. Average seasonal (spring vs. summer) and diurnal (morning vs. afternoon vs. nighttime) flux rates were compared with Mann-Whitney U tests for nonparametric data. Average methane fluxes from each treatment for spring and summer combined were also compared with Mann-Whitney U tests.

Carbon dioxide flux rates were analyzed according to daytime CO<sub>2</sub>-C uptake and nighttime CO<sub>2</sub>-C efflux for each treatment, averaged over the study period. Data were tested for normality and homogeneity of variance with one-sample Kolmogorov-Smirnov tests and the Levene statistic,

Table 1. Physical and chemical characteristics of each soil type used in the mesocosm experiment.

	Munsell profile*	Redoximorphic features	Db‡	%SOM	%C	%N
Hydric soil treatments	10YR 4/3 – 10YR 3/1 82% with chroma $\leq$ 2	Mottles $>$ 5% 5YR 4/5 – 10YR 5/8	2004: 1.25 $\pm$ 0.02 2005: 0.56 $\pm$ 0.01	4.97 $\pm$ 0.09	1.56 $\pm$ 0.06	0.15 $\pm$ 0.01
Non-hydric soil treatments	10YR 4/3 – 10YR 3/2 91% with chroma $\geq$ 3	Mottles $\leq$ 2% 10YR 6/7 – 10YR 6/8	2004: 1.29 $\pm$ 0.02 2005: 0.42 $\pm$ 0.01	4.96 $\pm$ 0.07	3.68 $\pm$ 0.04	0.16 $\pm$ 0.00

\* Hue (e.g. 10YR), value (#/), chroma (/#)

‡ Bulk density ( $\pm$  standard error) after soil settled, before and one year after mesocosms were flooded

respectively, and mean CO<sub>2</sub>-C uptake rates among treatments were compared with one-way ANOVA using Tukey's post-hoc test. Nighttime CO<sub>2</sub>-C efflux data were normally distributed with non-homogenous variances ( $p=0.012$ ), and were compared first with ANOVA, and treatments that had  $p$  values  $<$  0.3 were compared with Mann-Whitney tests. All analyses were performed in SPSS 11 for Mac.

Dominant plant species were determined for each treatment using the 50/20 rule (USACOE, 1987) applied as follows: 1) mean percent cover of each species for all sampling dates was obtained and multiplied by the number of sampling dates on which the species was observed; 2) total percent cover for all species was summed and multiplied by 50% (0.5) and 20% (0.2); 3) the species with the greatest percent cover were added up until the value obtained for 50% of the total percent cover was reached; these species were the dominants in a given treatment. No single species in any treatment had a percent cover value equal to or greater than the 20% value.

## Results

### Soils

#### Hydric characterization

Munsell color chart analysis of the soils, performed after filling and prior to flooding the mesocosms, verified hydric and nonhydric characteristics for the two source soils. Non-hydric source soils generally had chromas of 3, with a few samples being recorded as chroma 2. Minimal redoximorphic features, such as mottles, oxidized pore linings or redox depletions, were observed in non-hydric soils. The majority of the hydric source soils had chromas of 1 or 2, but a few samples were determined to have chromas of 3 or 4. Hydric soils contained substantial ( $>5\%$ ) redoximorphic features, mottles being the most frequently observed (Table 1).

#### Bulk density, carbon, nitrogen, and SOM

The bulk densities of each soil type after filling the tubs and prior to inundation were comparable (averaging 1.25 $\pm$ 0.02 and 1.29 $\pm$ 0.02 g cm<sup>-3</sup> for hydric and non-hydric soils respectively,  $p = 0.64$ ). Total carbon in non-hydric soils prior to inundation was significantly greater ( $p=0.05$ )

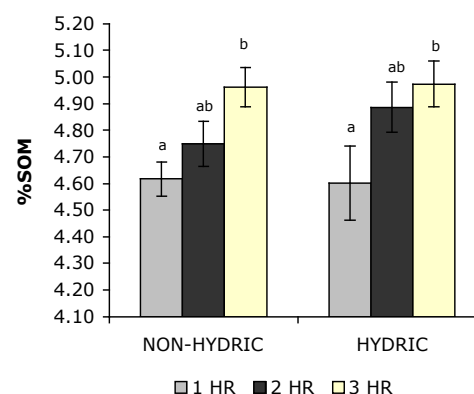


Figure 2. Percent soil organic matter (SOM) according to number of hours combusted at 550°C. Different letters indicate a significant difference in SOM between combustion times. Bars represent standard error.

than that of hydric soils (1.56 $\pm$ 0.06 and 3.68 $\pm$ 0.04 %C respectively), while total nitrogen content was nearly identical ( $\sim 0.16\%$ N, Table 1). Percent organic matter before inundation was also identical for hydric and non-hydric soils: 4.97 $\pm$ 0.09 and 4.96 $\pm$ 0.07% respectively ( $p=0.30$ ). Time of combustion made a significant difference for both hydric and non-hydric soils (Figure 2). While combustion for 2 hours resulted in higher SOM values than combustion for one hour, the differences were not significant ( $p=0.1$  and  $p=0.2$  for hydric and non-hydric soils respectively). Combustion for 3 hours (vs. 1 hr) produced significantly higher SOM values for both soil types ( $p=0.03$  and  $p=0.00$  respectively). Two vs. three hours of combustion made little difference for hydric soils ( $p=0.5$ ) but was close to significant for non-hydric soils ( $p=0.07$ ). Treatment with hydrochloric acid for detection of carbonates produced abundant fizzing in all of the non-hydric soils, but no fizzing in hydric soils. The greater %C in non-hydric soils was thus attributed in part to carbonates.

#### Nutrients in porewater

Nitrate-nitrite and ammonia concentrations were below the detection limit (0.002 and 0.003 mg N L<sup>-1</sup> respectively) in all porewater samples. Total phosphorus in porewater ( $\pm$  standard error) from hydric and non-hydric mesocosms were 86 $\pm$ 9 and 95 $\pm$ 22  $\mu$ g L<sup>-1</sup> respectively ( $p=0.67$ ), compared

Table 2. Plant species identified in each treatment (wetland indicator status in parentheses) during the gas sampling period.

	Hydric Pulsed	Non-hydric Pulsed	Hydric Steady-flow	Non-hydric Steady-flow
<b>Emergent wetland species</b>				
<i>Schoenoplectus tabernaemontani</i> K.C. Gmel (OBL)	x	x	x	x
<i>Sparganium eurycarpum</i> Engelm. (OBL)	x	x	x	x
<i>Eleocharis</i> spp (OBL)	x	x	x	x
<i>Leersia oryzoides</i> L. Sw. (OBL)	x	x	x	x
<i>Typha latifolia</i> L. (OBL)	x	x	x	x
<i>Carex</i> spp (FACW)	x	x	x	
<i>Eupatorium perfoliatum</i> L. (FACW+)	x			
<i>Lycopus americanus</i> L. (OBL)	x	x	x	x
<i>Mimulus ringens</i> L. (OBL)	x	x	x	x
<i>Asclepius incarnata</i> L. (OBL)			x	
<i>Verbena hastata</i> L. (FACW+)	x			x
<b>Submerged and floating plant species</b>				
<i>Algae</i> spp. (OBL)	x	x	x	x
<i>Ludwigia palustris</i> (L.) Elliott (OBL)	x	x	x	x
<i>Potamogeton</i> spp (OBL)	x	x	x	x
<b>Herbaceous upland species</b>				
<i>Solidago</i> sp.		x		
<i>Echinochloa crusgalli</i> L. Beauv. (FACU)		x		
<i>Plantago major</i> L. (FACU)	x	x	x	x
<i>Taraxacum officinale</i> Weber ex. Wiggers (FACU)	x	x		x
<i>Hibiscus trionum</i> L. (UPL)		x		x
<i>Trifolium repens</i> L. (FACU-)		x		x
<b>Woody species</b>				
<i>Acer negundo</i> L. (FAC+)		x		
<i>Salix nigra</i> Marshall (FACW+)	x	x	x	x
<i>Populus deltoides</i> Bertram ex Marshall (FAC)	x	x	x	x
<i>Acer rubrum</i> L. (FAC)	x	x	x	x

to a mean concentration of  $47 \pm 4 \mu\text{g L}^{-1}$  in groundwater samples. Soluble reactive phosphorus concentrations were  $18 \pm 5$  and  $13 \pm 5 \mu\text{g L}^{-1}$  in hydric and non-hydric porewater samples respectively, and  $18 \pm 13 \mu\text{g L}^{-1}$  in groundwater samples. Porewater samples extracted in June and August of 2005 contained negligible dissolved organic carbon; dissolved carbon was predominantly to entirely in inorganic form. Inorganic C contents of porewaters from non-hydric and hydric soils were not significantly different on either sampling occasion, with respective values of  $114 \pm 4$  vs.  $125 \pm 4 \text{ mg L}^{-1}$  on June 2 ( $p=0.49$ ), and  $135 \pm 12$  vs.  $136 \pm 14 \text{ mg L}^{-1}$  on August 4 ( $p=0.46$ ). Inorganic C content in groundwater samples taken on the same dates averaged  $84.23 \pm 1.08 \text{ mg L}^{-1}$ .

### Water soluble extractable organic matter

Although the content of cold and hot water extractable dissolved organic matter was similar for hydric and non-hydric soils, significantly greater quantities of each fraction were extracted from hydric soils (Figure 3). Cold water extractions produced  $17.7 \pm 0.7$  and  $15.4 \pm 0.6 \text{ g DOM per kg dry soil}$  for hydric and non-hydric soils respectively ( $p=0.02$ ). Hot water extractions produced  $28.5 \pm 1.2$  and  $24.0 \pm 0.7 \text{ g DOM per kg dry soil}$  for hydric and non-hydric soils respectively ( $p<0.01$ ). Duplicate extractions produced experimental results that were not significantly different

according to paired t-tests (CWEOM  $p = 0.99$ , HWEOM  $p = 0.74$ ).

### Vegetation

Twenty-three plant species were identified in the combined treatments, and a minimum of 16 species was identified in each treatment (Table 2). Percent cover was dominated by obligate wetland species in all treatments, but

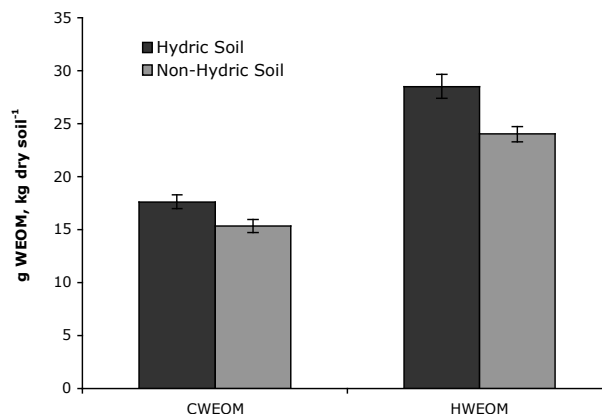


Figure 3. Cold-water extractable organic matter (CWEOM) and hot-water extractable organic matter (HWEOM) for hydric and non-hydric soils, measured one year after mesocosms were inundated.

Table 3. Dominant plant species in each treatment, determined using the 50/20 rule (wetland indicator status in parentheses).

Hydric Pulsed	Non-hydric Pulsed	Hydric Steady-flow	Non-hydric Steady-flow
<i>Potamogeton</i> spp. (OBL)	<i>Eleocharis</i> spp (OBL)	<i>Eleocharis</i> spp (OBL)	<i>Algae</i> spp (OBL)
<i>Eleocharis</i> spp. (OBL)	<i>Trifolium repens</i> L. (FACU)	<i>Algae</i> spp (OBL)	<i>Eleocharis</i> spp (OBL)
<i>Schoenoplectus tabernaemontani</i> K.C. Gmel (OBL)*	<i>Typha latifolia</i> L. (OBL)	<i>Potamogeton</i> spp (OBL)	<i>Schoenoplectus tabernaemontani</i> K.C. Gmel (OBL)*
<i>Leersia oryzoides</i> L. Sw. (OBL)*			

the non-hydric pulsed treatment contained a FACU clover species (*Trifolium repens* L.) as a dominant. *Eleocharis* spp (spikerushes) were dominant in all treatments, despite not being planted. No treatment had more than four dominant species, and both emergent and submerged (*Algae* spp.) or floating (*Potamogeton* spp.) vegetation were dominant in all treatments except for the non-hydric pulsed tubs in which no dominant species were submerged or floating. Woody plants occupied a small percentage of total cover in all treatments except for hydric steady-flow tubs, where they were virtually absent. The two treatments with non-hydric soils contained a greater number of upland plant species, but only slightly fewer emergent wetland species, and also contained floating and submerged plants. Of the three planted species, none was dominant in the treatments with non-hydric soils, and *Sparganium eurycarpum* Engelm was not dominant in either treatment with hydric soils (Table 3).

## Hydrology

Water depth in steady flow mesocosms averaged  $4.0 \pm 0.09$

cm for the duration of the experiment, while the water depth in pulsed tubs varied from 5 to  $-30$  cm (Figure 4). With the exception of April and May, pulsed tubs experienced at least two cycles of drawdown and inundation each month. The amount of water delivered to the mesocosms varied between  $150 - 700$  L month<sup>-1</sup>, with less water delivered during months with higher volumes of precipitation. The amount of water delivered to pulsed and steady-flow tubs was not significantly different ( $\alpha = 0.05$ ), except during May, when pulsed tubs received about 50 L more than steady flow tubs ( $p < 0.01$ ).

## Methane flux

### Hydric vs. non-hydric soils

Treatments containing hydric soils emitted significantly more methane than treatments containing non-hydric soils, in both spring and summer ( $p < 0.001$ ). Pulsed and steady-flow mesocosms with non-hydric soils together emitted an average ( $\pm$  standard error) of  $0.11 \pm 0.03$  and  $0.40 \pm 0.08$  mg m<sup>-2</sup> h<sup>-1</sup> during spring and summer respectively, while pulsed

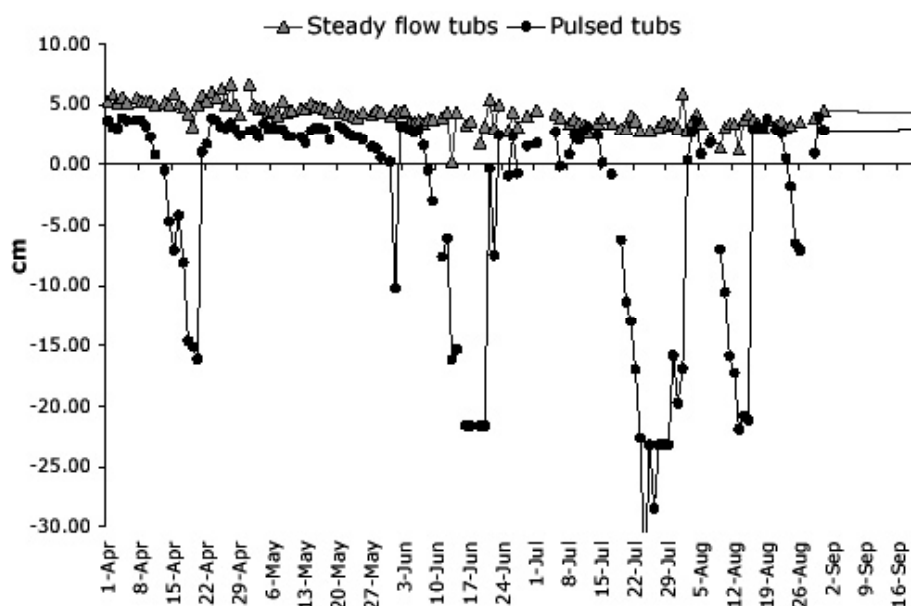


Figure 4. Daily water depths in steady-flow and pulsed tubs over the six-month gas sampling period. Negative values indicate water levels below the soil surface.



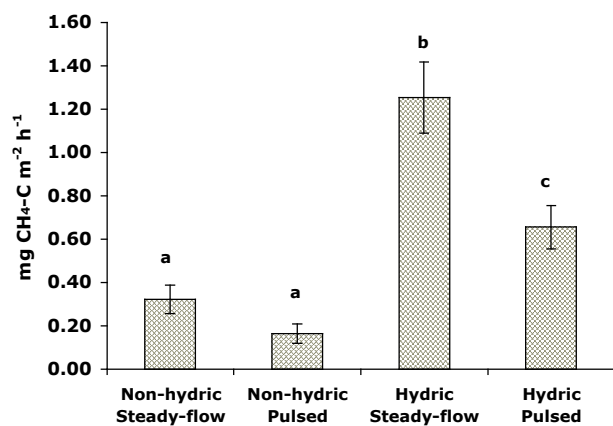


Figure 5. Mean methane emissions over the entire study period for each treatment. Number of samples is as follows: non-hydric steady flow: 214; non-hydric pulsed: 171; hydric steady-flow: 176; hydric pulsed: 180. Bars represent standard error.

and steady-flow mesocosms containing hydric soils together emitted  $0.72 \pm 0.09$  and  $1.16 \pm 0.17$   $\text{mg m}^{-2} \text{h}^{-1}$  during spring and summer respectively. Analyzing each treatment separately over the entire study period, methane fluxes ranged from  $0.17 \pm 0.04$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$  from non-hydric pulsed tubs, to  $1.25 \pm 0.16$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$  from hydric steady-flow tubs. Fluxes were significantly different ( $p < 0.003$ ) between all treatments except for non-hydric steady flow vs. non-hydric pulsed mesocosms ( $p = 0.243$ , Figure 5).

### Time of day

There were no significant differences ( $\alpha = 0.05$ ) in methane flux according to time of day, within treatment types. Temporal differences between treatments were observed. Steady-flow mesocosms with hydric soil emitted significantly more methane than all other treatments at all times of day ( $p < 0.02$ ). Pulsed mesocosms with hydric soil emitted a greater quantity of methane than steady flow mesocosms with non-hydric soil, but the difference was only significant at night ( $p = 0.05$ ). Comparing pulsed treatments with hydric vs. non-hydric soil, tubs with hydric soil emitted significantly more methane ( $p < 0.05$ ) than those with non-hydric soil in the afternoon and at night, but not in the morning ( $p = 0.09$ ) (Figure 6).

### Hydrology

There was more variability in methane flux from pulsed tubs with hydric soils than from steady flow tubs with hydric soils. The former exhibited rising and falling rates of  $\text{CH}_4$  emission that lagged changes in hydrology by approximately one week. Methane flux from each treatment will be discussed individually in relation to hydrology:

**Hydric pulsed:** After 25 days of inundated conditions from mid May-mid June, methane flux approached the maximum observed in the hydric pulsed treatment ( $\sim 2$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ). The highest rates of methane flux occurred after mesocosms had been flooded continuously for at least 3 weeks. A two-week dry down occurred in mid-June, during which methane emissions dropped to  $< 0.5$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$ . Three shorter intervals (max 2-weeks each) of flooding/dry

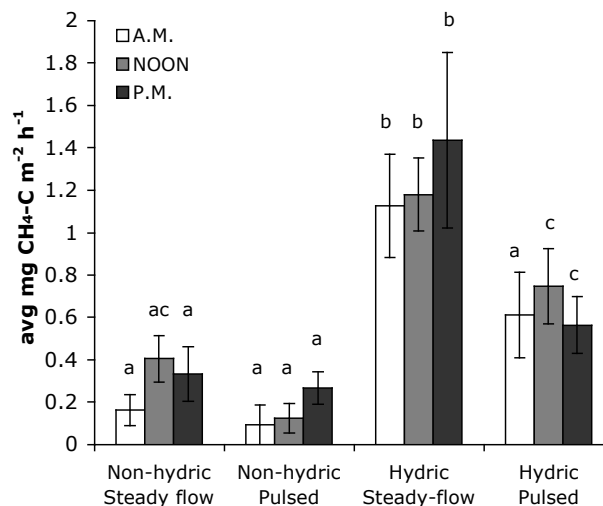


Figure 6. Mean methane flux for each treatment according to time of day. Different letters indicate a significant ( $p \leq 0.05$ ) difference among treatments. Bars represent standard error.

down occurred between July 19-mid September. Methane flux from hydric pulsed mesocosms during these shorter periods of inundation was minimal. (Figure 7A).

**Non-hydric pulsed:** The hydrologic pattern in non-hydric, pulsed mesocosms was roughly identical to that of hydric tubs, however, methane flux showed no apparent relationship to the dynamic hydrology. Average methane emission from non-hydric pulsed tubs fluctuated around a narrow range ( $-0.2$  to  $0.5$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ). Methane flux remained steady at the average rate for non-hydric pulsed mesocosms even when the tubs experienced a two-week dry down (Figure 7B).

**Hydric and non-hydric steady-flow:** Hydric, steady-flow mesocosms demonstrated a less variable pattern in methane flux than hydric pulsed mesocosms, with the highest fluxes (up to  $5.3$   $\text{mg CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ) occurring from late June through early August. Methane flux rates were the most variable from late July through September (Figure 7C). Methane flux from non-hydric steady flow mesocosms was more variable than that from non-hydric pulsed mesocosms. The greatest variability in flux from non-hydric steady-flow tubs occurred between May 31 – June 30 (Figure 7D).

### CO<sub>2</sub>-C uptake and efflux

No relationship was observed between rates of methane flux and daytime  $\text{CO}_2$  uptake for any treatment type ( $R^2 < 0.02$  for all treatments), and relationships between methane flux and nighttime  $\text{CO}_2$  efflux were similarly weak ( $R^2$  between  $0.02 - 0.09$  for the four treatments). Rates of  $\text{CO}_2\text{-C}$  uptake were not significantly different ( $p = 0.80$ ) between spring (sampled Apr 6 - June 14) and summer (sampled June 21 - Sept 20), although they were higher in summer. Likewise,  $\text{CO}_2\text{-C}$  efflux was not significantly different between the seasons ( $p = 0.06$ ), but was higher in summer (Figure 8). Over the course of the entire study, the highest average rates of  $\text{CO}_2\text{-C}$  uptake ( $467 \pm 18$   $\text{mg m}^{-2} \text{h}^{-1}$ ) occurred in the non-hydric steady flow treatment, followed



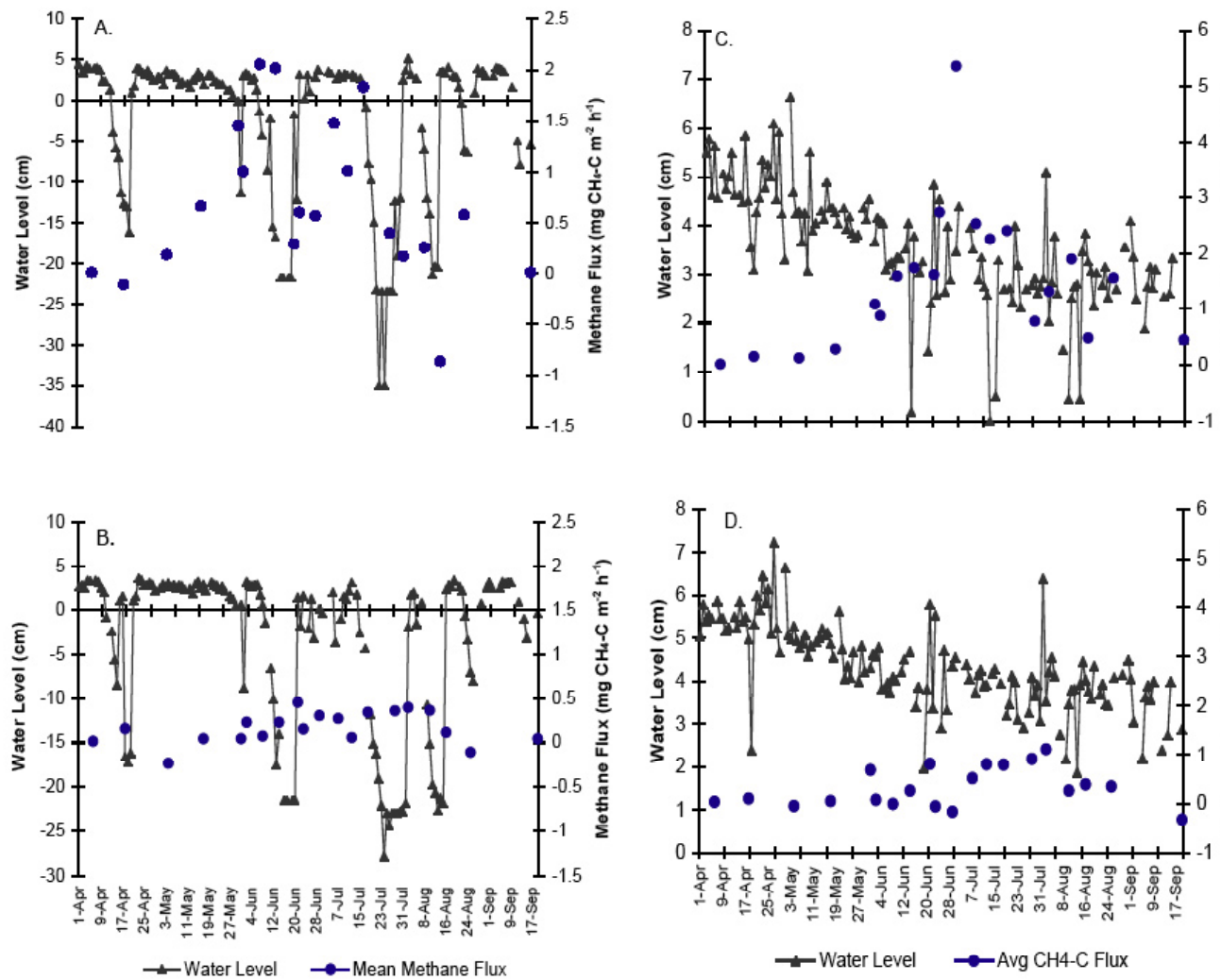


Figure 7. Mean daily water level, and mean diurnal methane flux on each sampling date for each treatment: A: Hydric pulsed; B: Non-hydric pulsed; C: Hydric steady-flow; D: Non-hydric steady-flow.

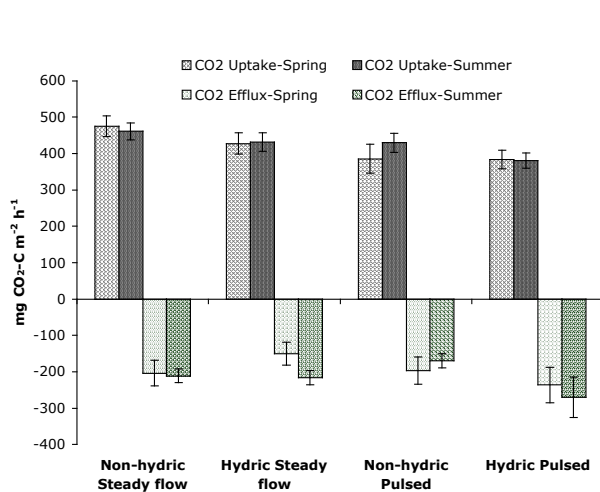


Figure 8. Mean rates of CO<sub>2</sub>-C uptake and efflux for each treatment in Spring (sampled Apr 6 - June 14) and Summer (sampled June 21 - Sept 20). CO<sub>2</sub>-C uptake was measured during morning and afternoon sampling periods, and CO<sub>2</sub>-C efflux was measured during nighttime sampling. Bars represent standard error.

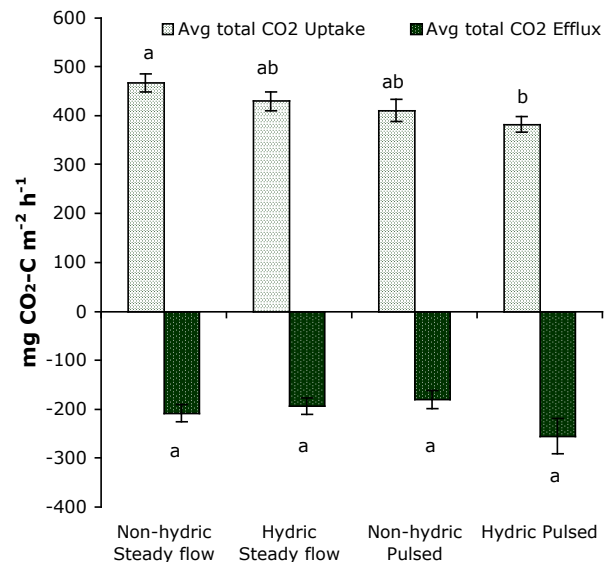


Figure 9. Mean rates of CO<sub>2</sub>-C uptake and efflux for each treatment over the entire gas-sampling period (April - September 2005). Different letters indicate a significant difference between treatments for either uptake or efflux.

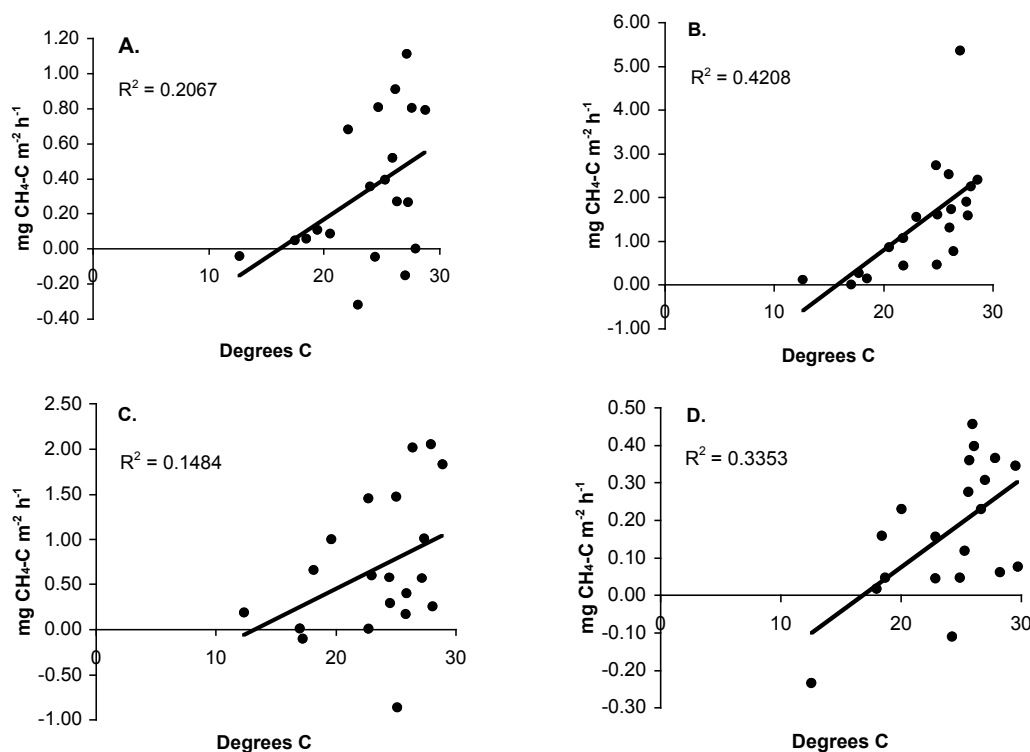


Figure 10. Correlations between soil temperature and methane flux for each treatment. A = Non-hydric steady flow; B = Hydric steady flow; C = Hydric pulsed; D = Non-hydric pulsed. Soil temperatures are averages of all measurements made for each treatment at 5 and 10 cm depth. Methane fluxes are averages of all flux rates (morning, afternoon, night) on each sampling date for each treatment.

by hydric steady-flow ( $429 \pm 19$  mg  $m^{-2} h^{-1}$ ), non-hydric pulsed ( $410 \pm 23$  mg  $m^{-2} h^{-1}$ ), and hydric pulsed ( $382 \pm 16$  mg  $m^{-2} h^{-1}$ ). Significantly less ( $p=0.006$ )  $CO_2$ -C uptake occurred in the hydric pulsed treatment compared to the non-hydric steady flow treatment. No other differences were significant. Rates of  $CO_2$ -C efflux did not differ significantly among any of the treatments (Figure 9).

### Soil temperature

At the start of gas sampling in April 2005, soil temperatures between 5-10 cm depth averaged 17-18°C for all treatments. Soils warmed consistently through mid July (27.5 - 29.5°C), after which temperatures began to decline again. There was one aberration in the warming trend, with mean soil temperatures of 12.3 - 12.7°C on the May 4 sampling date. At the end of the study in late August, mean soil temperatures were 21.8 - 22.9°C. Regressions between soil temperature and methane flux indicated that soil temperature explained between 15 and 42% of  $CH_4$ -C flux in treatments with hydric soils, and between 21 and 34% of  $CH_4$ -C flux in treatments with non-hydric soils (Figure 10).

## Discussion

### Soil physical properties

The percent carbon content of the hydric soils was surprisingly low, equal to the %C value of the ORWRP

surface soils prior to flooding (Nairn, 1996). Despite efforts to remove only the upper 10 cm of soils from the Billabong, such precision was difficult to achieve with the backhoe, and it is likely that some of the underlying soils were removed as well. However, the lack of variation in %C and SOM values for hydric mesocosms implies consistency in the soils used for the hydric treatments. Despite higher %C, non-hydric soils contained the same amount of SOM as hydric soils, and the observation of carbonates in the non-hydric soils lead us to attribute the difference in %C to carbonates. Combustion of soil or sediment samples at temperatures between 360°C (Konen et al., 2002) and 550°C (Smith, 2003; Anderson et al., 2005) is a standard practice for determining organic matter content without volatilizing carbonates, which occurs at temperatures above 900°C (Heiri, 2001). We found the greatest correlation for duplicate soil samples ( $n=9$ ) combusted for 3 hours ( $R^2=0.99$ , compared with  $R^2=0.86$  and  $R^2=0.90$  for 1 and 2 hours respectively), and given the significant difference in SOM according to combustion time, it can be concluded that three hours will provide more reproducible and accurate results than one-hour combustion.

Numerous researchers have demonstrated correlations between hot water extractable carbon, labile carbon and microbial biomass (Sparling et al., 1998; Ghani et al., 2003; Jinbo et al., 2006). Specific microbial processes, including denitrification potential and anaerobic mineralizable carbon (Hernandez and Mitsch, in press) have been positively

correlated with CWEOM. In this study, CWEOM and HWEOM from non hydric soils were 88 and 85% of that from hydric soils, much less than the difference in methane fluxes from non-hydric vs. hydric treatments. WEOM extractions were performed on all soils after one year of maintaining saturated hydrology and establishing vegetation. It is possible that the values for WEOM may have been different at the end of the second growing season and the gas sampling period. However, the microbial biomass can consist of diverse populations, not just methanogens.

## Vegetation

Both hydric and non-hydric soils supported a diverse plant community, but none had more than four dominant species. While this could be attributed in part to the small volume:surface area ratio of the mesocosm tubs, full scale wetlands at the same research site have generally been dominated by four or fewer species, despite being colonized by a diversity of species (Mitsch et al., 2005). The majority of herbaceous species in each treatment were FACW or wetter status, indicating that these species occur in wetlands 67–99% of the time (Reed, 1988). The fact that only two of the six UPL or FACU species were observed in hydric soil treatments suggests that either the hydric soil seedbank contained fewer upland plant propagules, or that upland species had been rendered unviable since establishment of the Billabong wetland in 1997. Not surprisingly, the greatest percentage of herbaceous upland species was found in the non-hydric pulsed treatment, where soils were only inundated during a portion of the study period. The highest species richness was observed in this treatment as well, but the greater species richness was due entirely to the presence of the six upland species.

Mesocosm studies enable isolation of parameters of interest for examination, with replication often not possible in ecosystem-scale studies. A central focus of this study was to examine the effects of flood pulses (cycles of inundation and dry down) vs. continuous inundation on methane and carbon dioxide fluxes in wetlands. Because they are contained systems, the mesocosms excluded floodplain effects and made it possible to examine effects of hydrology independent of variables that would have been introduced if the water had traveled across a floodplain, including extra nutrients, contaminants, plant or animal propagules and sediments.

## Methane flux in relation to hydrology, soils, time of day and CO<sub>2</sub> fluxes

Both soil type and hydrology were important in determining differences in methane flux between the four treatments, and hydrology was especially important in determining methane flux from treatments with hydric soils. The low rates of methane emissions from non-hydric treatments are still within the range reported for floodplain wetlands in ecosystem studies. Boon et al. (1997) documented fluxes of  $0.1 \pm 0.02$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> to  $0.7 \pm 0.2$

mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> from Ryans 5 Billabong, Australia, after two separate flood events. In a related mesocosm experiment, these researchers found that methanotrophy appeared to dominate the methane budget; despite prolonged periods of inundation, methane produced in mesocosm sediments did not reach the atmosphere (Boon et al., 1997).

Rates of CH<sub>4</sub> flux from mesocosms with hydric soils were comparable to or lower than average rates reported in the literature for other floodplain wetlands. In Venezuela's Orinoco River floodplain, areas with macrophytes emitted  $0.78 \pm 0.04$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>, and forested areas emitted  $3.40 \pm 0.04$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> during flooded conditions (Smith et al., 2000). During low water conditions in the Amazon floodplain, areas containing aquatic macrophytes produced average fluxes of  $3.83 \pm 0.14$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>, while during high water such areas produced  $10.13 \pm 0.28$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> (Melack et al., 2003). Experimental wetlands located at the ORWRP that were inundated for 12 years emitted  $3.40 \pm 0.47$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> in intermittently flooded areas, and  $7.66 \pm 1.78$  mg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> in continuously inundated areas (Altort and Mitsch, in press). The organic matter content of the experimental soils in this study was comparable to that measured in surface soils from the same site in 1993, before the experimental wetlands were inundated (Anderson and Mitsch, 2005). The relatively low methane flux rates measured in the mesocosms can be attributed in part to low levels of organic matter accretion in the soils, a parameter that also reflects in part the amount of microbial biomass present in the soils.

The lack of diurnal variation in methane flux supports our observation at the ecosystem scale that emergent macrophytes do not appear to be conducting CH<sub>4</sub> to the atmosphere via pressurized ventilation in created wetlands. While this mechanism has been well-described by numerous other researchers for individual plant species and natural wetland ecosystems with standing water, it has not been convective flow of methane through vascular plant tissue has not been reported extensively for created wetlands, especially those with a fluctuating water table. Similarly, the highest rates of CO<sub>2</sub> uptake were observed in the non-hydric steady-flow treatment, a treatment exhibiting low rates of methane flux. The lowest rates of CO<sub>2</sub> uptake were observed in hydric-pulsed treatment, where rates of methane flux were higher. Although the CO<sub>2</sub> fluxes reported here do not represent comprehensive rates of photosynthesis or respiration, they do illustrate general trends in productivity of the four treatments. Productivity and organic carbon accumulation could be expected to increase in systems receiving a higher nutrient load. Although unplanned, the use of groundwater in this study enabled an examination of the parameters of interest independent of variables that would be introduced in river water traveling across a floodplain, including nutrients, contaminants, biota and sediments.

## Conclusions

The results of this study suggest that wetlands created

on non-hydric soils will emit modest quantities of methane, and CH<sub>4</sub> flux rates will increase with time as hydric soil characteristics develop. Establishment of dynamic hydrology such as characterizes natural floodplain wetlands may help to minimize methane fluxes as the created wetland ages. Flood-pulse hydrology may initially make little difference in terms of methane flux, but may facilitate establishment of a diverse plant community. Created wetlands located in landscapes that are open to propagule introduction, for example by river flooding, waterfowl or mammal use, may not need to be seeded or planted with macrophytes. Planting is costly in terms of dollars and effort, and in many cases may not be necessary. Only one of the three macrophyte species planted in this study became dominant, and it was only dominant in half of the treatments. Finally, based on the time trial conducted for soil organic matter determination, it is recommended that soils be combusted for a minimum of three hours for calculations of SOM by loss on ignition. However, it is recommended that combustion be conducted in intervals that enable comparison with previous studies conducted at this or other research sites.

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